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Secrets of a cell's transformation to a cancerous state are coming to light through investigations into the nature of oncogenes. Gail E. Sonenshein, Ph.D., in the BUSM Department of Biochemistry, is studying the way *myc*—one of 20 oncogenes—operates. See story on page 3.

Researchers find probable cause of autism; may solve 40-year puzzle

Months of painstaking research has allowed a Boston University School of Medicine researcher to find what probably is the solution to one of the "kingpuzzles in pediatric neurology"—the cause of autism.

Thomas L. Kemper, M.D., a professor of neurology (neuropathology) and anatomy at BUSM, along with co-investigator Margaret Bauman, M.D., of Massachusetts General Hospital, has found a brain abnormality that may help to explain the disorder. Scientists have been struggling to find the cause of autism, a baffling condition in which children withdraw into their own private world, since it was recognized as a syndrome in 1943.

The researchers' results, if confirmed, will allow other scientists to consider new ways to study, and possibly to treat, autism.

According to Kemper, these findings also take a tremendous emotional burden off parents of autistic children who have been told their children are autistic because the children did not receive enough emotional stimulation when they were babies. This was the prevailing theory on the cause of autism until the mid-1970s, and scientists have not had hard evidence to dispute this

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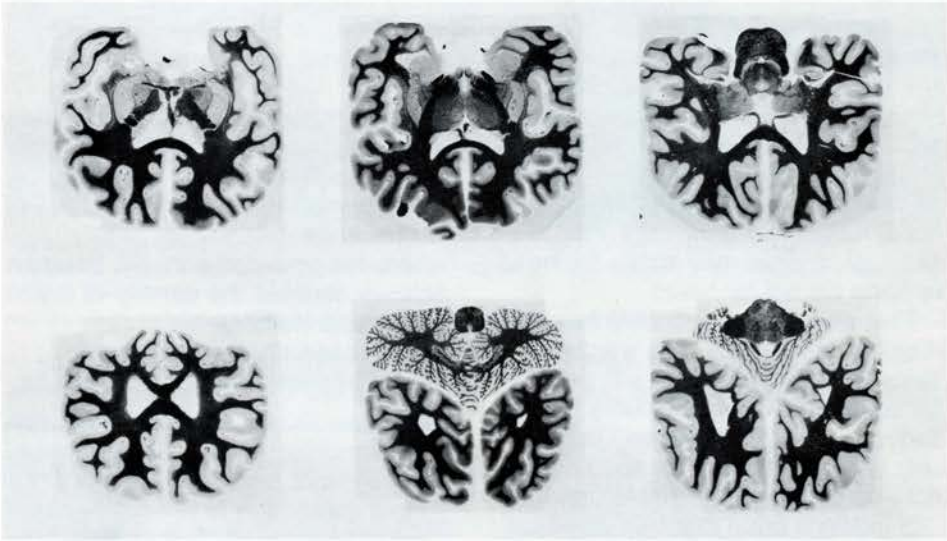

A painstaking study of a brain cut into more than 3,000 slices, some of which are pictured here, has allowed a BUSM researcher to find defects that may explain the cause of autism. (Photo by Bradford F. Herzog)

20-year VA study of 2,000 males examines unknowns of aging process

Everyone has seen an elderly relative or friend suffer from various age-related ailments: increasing physical infirmities, lowered resistance to disease, the weakening or possible loss of mental function. But until recently, the medical community has focused most of its attention on the problems of pathological, or non-normal, aging. The factors and processes that influence normal aging have been left largely unexplored.

A large interdisciplinary study headed by Boston University School of Medicine researcher Pantel S. Vokonas, M.D., may help to solve many of the unknowns attached to normal human aging.

The Veterans Administration Normative Aging Study (NAS), which began in 1963, has followed continuously the progress of 2,000 initially healthy men from the Greater Boston area. The study, funded by the VA with additional support from the National Institutes of Health, was undertaken to determine the normal patterns of aging and the precursors of diseases of aging, as well as the influence of these diseases on the aging process, according to

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theory until now.

"I don't know how many mothers I've talked to who were relieved to know that they did nothing to cause the disorder in their children," said Kemper, who also is director of neuropathology at Boston City Hospital, a principal teaching hospital of BUSM.

Autism affects four or five of every 10,000 children; males outnumber females four to one. Autisic children often develop normally through their first year of life. They then withdraw from others; they do not learn language and often engage in monotonous, repetitious behaviors. For example, a child may shake his head or bang his fist for hours.

Though Kemper, a BUSM faculty member since 1975, has wanted to study the brain of an autistic person for some time, he had trouble both finding a qualified co-investigator with the time needed for such a project and obtaining a suitable brain.

"Finding a brain that has sufficient documentation to satisfy the scientific community that you actually are studying a brain of an autistic person is difficult. Also, it's not a fatal disease, so there are not many brains available," explained Kemper.

Finally, through N. Paul Rosman, M.D., a professor of pediatrics and neurology at BUSM, Kemper acquired the brain of an autistic man. The brain was cut into more than 3,000 slices, each only 35 microns thick (about one-twentieth as thick as a human hair). The brain of a man without the disorder was cut in exactly the same manner to use as a comparison.

Kemper then teamed with Bauman, an assistant neurologist at MGH, and the two began the yearlong task of comparing the two brains.

"We went through the entire brain. We essentially looked at each of the hundreds of identified areas of the brain," said Kemper.

In their study the researchers used a special microscope that enabled them to see exactly the same area of both the autistic brain and the "normal" brain side by side, making for more accurate comparison.

"If you just look at the sections with your bare eye, you see very little. And even if you look at them under the microscope, without an exact comparison you see very little," said Kemper. "That's probably why people didn't see the defect in the past."

When the researchers found areas in the autistic brain they thought differed from the "normal" brain, Bauman counted the cells in those areas to check the differences. "That's where the time came in. Dr. Bauman actually counted the density of the cells," said Kemper.

The researchers also did cell counts in areas that appeared to be normal, but were suspected of being related to autism.

Thomas L. Kemper, M.D., shown here examining brain slices, has found a brain abnormality that may help to explain the cause of autism. (Photo by Bradford F. Herzog)

Through this tedious examination, Kemper and Bauman found two areas of the autistic brain in which there were abnormalities—the limbic system and the cerebellum, located in the base of the brain.

In the limbic system, abnormalities were spotted in the amygdala, the hippocampus and areas closely related to them. These areas of the brain control memory, emotion and motivation.

"The cells were abnormally small and abnormally tightly packed together. It gives the appearance of a failure of normal development or a brain of a much younger person. Exactly what age it would correspond to, we don't know yet," said Kemper.

In the cerebellum, the brain area that controls coordination of movement, there was a loss of nerve cells, said Kemper. "The form of the defect in the cerebellum allows us to conclude that this abnormality very likely developed before 30 weeks of gestation, well before birth," he explained.

Kemper said behaviors controlled by these abnormal areas, especially memory, seem very closely related to the problems of autistic children.

"The clinical defects are in interpersonal relationships, memory and abnormal behaviors. The anatomical defects found in the brain are right in the middle of the memory circuits. When you start thinking about memory and human behavior, then you're into language and recognizing people. That may be the core of the problem."

Of the two treatments commonly used on autistic children—behavioral modification and medication—behavioral modification is by far the more effective, according to Kemper. Using this, medical personnel can work at teaching the children after modifying their abnormal behaviors. Medication, on the other hand, often merely masks or blunts the symptoms.

Kemper explained, though, that while behavioral modification can be effective, it is also very time-consuming and thus very costly. "We've followed some patients for more than 10 years, testing them at periodic intervals on such skills as speech and language, IQ scores and mental ages. The striking aspect we have found is that they progress at a snail's pace," he said.

Kemper hopes other researchers will be able to use these findings to develop better treatments for people with autism.

"These findings open up autism for more experimental research. One can start using animal models of autism and start 'going after' the disorder with various treatment modes for manifestations of the disease to see which treatments are most effective. This is very important."

Kemper recently acquired the brain of an autistic 10-year-old girl through the McLean Brain Bank, McLean Hospital, Belmont, Mass., and has applied to the National Institutes of Health and the Stallone Foundation for funds to process this brain. He said that if studies of three or four more autistic brains reveal brain defects similar to those he and Bauman found, then he will be satisfied with the validity of the findings.

"The knowledge of what causes autism will allow us to think about how the disease could have come about. Hopefully this will allow scientists to recognize similar diseases and see where they fit in the spectrum of abnormal brain development. That's one of the most gratifying things about this work," said Kemper.

— Paul D. Vaskas

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What makes *myc* tick? Researchers study oncogene's role in cancer development

When oncogenes first were discovered more than a decade ago, many researchers saw them as foreign elements that infiltrated a person's genetic code through viral infection, then altered normal cell function and caused tumors.

Since then, oncogene research has progressed rapidly, producing many exciting surprises. One thing now is clear: oncogenes are not necessarily outside invaders. Proto-oncogenes exist within the normal genetic makeup of almost all cells—animal or vegetable. These genes are fundamental and their persistence throughout evolution suggests they play a crucial role in normal cell growth and development. Mutation or other alteration of a proto-oncogene can result in its conversion to a cancer-causing oncogene. Scientists now believe that, under certain circumstances, oncogenes—either by themselves or in conjunction with other genes—disrupt normal cell function and control of cell proliferation.

What causes some cells to express or activate an oncogene, and what changes occur in the gene itself or in its regulation to make it dangerous, are questions being addressed by Gail E. Sonenshein, Ph.D., and her colleagues in the Boston University School of Medicine Department of Biochemistry. Such basic research, said Sonenshein, will aid in understanding how cells control their growth and will be of use in developing new therapies for cancer victims.

Scientists have identified at least 20 different oncogenes so far, some of which appear to be associated with particular forms of cancer. Sonenshein and her research associates are focusing their studies primarily on one oncogene, known as *myc*, most often associated in humans and mice with the development of certain tumors of the lymph tissue. The *myc* proto-oncogene has been found expressed in all growing cells tested by the BUSM researchers so far.

While most of Sonenshein's studies have involved mouse cells, plans are under way, in conjunction with other investigators at Boston University Medical Center, to extend current research to human tumor cells in an effort to develop new therapies for lymphoma patients.

Sonenshein, an associate professor of biochemistry, began studying *myc* as a result of her research on the control of B-cell antibody production. (B-cells primarily are responsible for producing antibodies that are the body's main defense against harmful elements.) In the course of her investigations using mutant B-cell lines, she discovered that a translocation event between a pair of chromosomes (an exchange of genetic material) caused a normally inactive antibody gene to become active.

According to Sonenshein, "The break occurred such that a new gene was aligned next to the normally silent antibody gene and that new gene turned out to be an oncogene."

Analysis of a number of different human and mouse B-cell tumors revealed, in many, a similar movement of the *myc* oncogene from one chromosome to another, implicating this translocation in the development of cancer.

As a result of this discovery, made simultaneously by several different groups, Sonenshein began investigating how *myc* causes a cell to become cancerous. Experiments from several labs seemed to eliminate the possibility of cancer caused simply by overproduction of the *myc* protein, and it was established that the products of the normal and translocated genes were the same. Therefore, the most likely remaining

Gail E. Sonenshein, Ph.D., examines data on oncogene activity with research assistant James E. McCormack. (Photo by Joan Clifford, Educational Media, BUSM)

possibility was that the normal mechanism regulating *myc* activity was somehow disrupted in cancer cells, causing this gene to be "on" at the wrong time.

"We decided to examine when *myc* goes on and when it goes off, and how a cell regulates this expression," said Sonenshein.

She and colleagues at Harvard Medical School already have established that when cultured, normal fibroblast cells stop growing, *myc* activity drops to barely detectable levels. When cells are actively growing, in the presence of naturally-occurring elements called growth factors, they maintain a certain level of *myc* activity. In chemically transformed fibroblast cells, even when growth is halted by withholding the necessary growth factors, *myc* remains nearly as active as if the cells were growing. The researchers concluded that the tumor cells have lost their ability to turn the *myc* gene off in a normal fashion. As a result, the change to a cancerous state may be due to *myc* being on at the wrong time during a cell's growth cycle.

Questions remain, however, as to whether the misregulation is within the *myc* gene itself or in some other gene capable of controlling *myc* expression. It is known, for example, that some growth factors can activate the *myc* gene. One possible explanation, then, for abnormal *myc* behavior in tumor cells is that cancer cells produce their own growth factors, causing *myc* to be continually active.

Experiments in conjunction with Judith Campisi, Ph.D., a BUSM assistant professor of biochemistry, are in progress to test whether or not another gene is making a growth factor-like substance that signals the oncogene to stay active. Preliminary results show that, in the absence of growth factors, one of the tumor cells tested continues to proliferate, suggesting it is receiving growth factor-like signals from within itself. Much more research is necessary, however, in order to understand the precise role of growth factors in *myc* regulation.

In related experiments, Sonenshein plans to use sophisticated molecular biological approaches and genetic engineering techniques to "dissect" the *myc* oncogene to determine more accurately where the control elements lie. "We will introduce cloned *myc* genes into cells. If we retain the normal control, then we can start to eliminate portions of the gene to determine which are the crucial control regions," she said.

Along with Thomas Rothstein, M.D., Ph.D., an assistant professor of medicine, Sonenshein is planning experiments with human tumor cells. Rothstein is treating cultures of patients' B-cells with antibodies to see whether or not the cells stop proliferating. He is finding that B-cells from some patients respond to antibody treatment in a manner similar to the way the mouse B-cells responded in earlier experiments, that is, they stop growing. However, he has some samples that do not respond to the treatment and others that even begin to grow more rapidly.

"We feel these differences may relate to alterations in the *myc* gene, and we currently are testing this possibility," said Sonenshein.

Other researchers who have been involved in the *myc* oncogene research are Ann Marshak-Rothstein, Ph.D., an assistant professor of microbiology at BUSM, former BUSM researchers Michael Dean, Ph.D., and Rachael B. Kent, Ph.D., and doctoral students Vincent H. Pepe and James E. McCormack. Sonenshein's research is supported by funds from the National Institutes of Health.

— Caroline H. Lupfer

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Aging study...

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Vokonas, the study's fourth director.

"We want to explain how a 30-year-old becomes a 70-year-old," said Vokonas, who is an associate professor of medicine at BUSM and a member of the Section of Preventive Medicine and Epidemiology. "We recognize many of the biomedical markers that characterize aging, but what we want to find out is how these processes work and how they interact with disease. There really is only limited information regarding the patterns of normal aging."

As might be expected from a study entering its 20th year, the NAS already has yielded much information. More than 150 articles and book chapters based on data from the study have been published in scientific and technical journals worldwide. For example, the findings have elicited substantial information about:

• *Cardiovascular disease*: Serial changes in ECGs can be predictive of higher rates of heart attack, ischemic heart disease and development of hypertension. Serial changes in blood lipids (cholesterol and triglycerides) over time are more predictive of ischemic heart disease than absolute values.

• *Physical anthropometry of the human body*: Repeated measurements of body dimensions have shown that a progressive decrease in height occurs with advancing age due to compression of the intervertebral spaces. Body weight increases until age 55 and decreases after age 65. Computerized tomographic scanning studies have demonstrated that this decrease is largely due to a decline in the amount of lean tissue (composed primarily of muscle), leading to an increase in the percentage of fat. Findings also have shown that the amount of fatty tissue is related to various diseases, including heart disease, and its distribution is indicative of the risk of diabetes.

Epidemiologist David Sparrow, M.A., in lab coat, administers a test of pulmonary function to Normative Aging Study participant William J. Callahan. (Photo by Lewis A. Glass, Educational Media, BUSM)

• *Smoking and pulmonary function*: One 10-year study has shown that the significant normal decline in pulmonary function in aging men is considerably greater in those who smoke. Change in pulmonary function for quitters of at least 10 years is much closer to that of those who have never smoked.

• *Alcohol consumption and behavior*: Ongoing studies of drinking behaviors have shown that social contexts for drinking are important predictors of alcohol consumption levels and the extent of problem drinking. Also, data have demonstrated no real decline over nine years in consumption levels or rates of problem drinking. Retirement seems to have little effect on drinking behaviors.

The NAS evolved from an earlier study of Spanish-American War Veterans, ages 80 through 90. The NAS participants are nearly all veterans who at the outset of the study were aged 20 through 81. They return to the VA Outpatient Clinic in Boston for medical examinations every five years (every three years after age 52). The mean age of the participants when the study began 20 years ago was 42; now it is approximately 60.

"The NAS has no defined endpoint," said Vokonas. "It extends for the lifetime of its participants. We want to examine the whole concept of aging as an essential developmental phenomenon of life, and ideally, be able to indicate which participants are aging normally and which will be more prone to disease than others."

According to Vokonas, the NAS uses two kinds of investigative mechanisms to gather necessary information about participants. Core studies involve all participants, and include a comprehensive physical history and examination, electrocardiograms, chest and hand films, pulmonary function measurements, tests for special senses such as eye and ear function, anthropometry (the measurement of different body dimensions) and a battery of biochemical tests.

Special projects involve selected groups within the study. "Special projects cover a range of subjects, from the strictly biomedical to the

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social and emotional," said Vokonas. "These projects allow us to take an in-depth look at a given function or activity." Special projects have ranged from a study that monitors changes in the relationship between fat and muscle in older adults, to ones that investigate the physical, psychological and social effects of such life changes as retirement.

According to Vokonas, the so-called longitudinal design of the NAS helps to give the study its unique perspective. Longitudinal studies examine the same subjects as they age over an extended period of time, as opposed to cross-sectional studies, which investigate a group of subjects of varied ages during a brief period of time.

"A simple example illustrates this difference," Vokonas explained. "We know that height decreases with advancing age, but also that a previous generation of men was shorter than they are now. Repeated measurements in the same individual over an extended period of time in a longitudinal study allow us to determine the decrement in height related to aging alone, and permits delineation of this from generational or cohort differences. In contrast, a cross-sectional study will reveal that height is lower in older individuals but cannot account for generational differences."

Another bonus of a longitudinal study, he said, is that its ability to focus on individuals rather than groups allows it to account for the extreme variability in health of older people. "The variation in health and disease gets much wider with aging—extraordinarily so," Vokonas said. "Test a group of 20-year-olds, and many of the tests will reveal a narrow range of results, but test a group of 70-year-olds, and there will be a much wider range of results for whatever parameter is tested."

In the future, Vokonas plans some minor modifications of the study's design. "As a clinician and cardiologist, I would like to see the study's biomedical domains strengthened. In addition, we will be expanding research concerning neuropsychological function and cognitive performance." Vokonas also is planning collaborative studies with other BUSM researchers, including colleagues from the BUSM Department of Medicine, School of Public Health and the University's Gerontology Center.

Vokonas said he hopes the study, in addition to providing a comprehensive profile of the aging process, will produce greater knowledge about how to achieve a more fulfilling life in the later years.

"We're all going to age anyway, whether we like it or not—we might as well adopt those measures now that will likely make those years happy and health ones."

Vokonas said he feels strongly about the ultimate value of the Normative Aging Study. "The VA predicts that there will be eight million veterans over the age of 65 by the year 2000—this is in contrast to three million currently. This organization alone may have to triple its resources to take care of these individuals. The greying of America is coming, and it's looming like a large cloud on the horizon bringing a host of problems concerning the care of elderly that must be addressed. There will be no end to the importance of this and other studies, or to these problems and solutions."

— Marilyn J. Davis

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